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<b>(21) International Application Number:</b> PCT/US94/02157 <b>(22) International Filing Date:</b> 9 March 1994 (09.03.94)  <b>(30) Priority Data:</b> 08/031,743 15 March 1993 (15.03.93) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 08/031,743 (CON) Filed on 15 March 1993 (15.03.93)  <b>(71) Applicants (for all designated States except US):</b> G.D. SEARLE & CO. [US/US]; Corporate Patent Department, P.O. Box 5110, Chicago, IL 60680-5110 (US). THE MONSANTO COMPANY [US/US]; 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> TJOENG, Foe, S. [US/US]; 875 Sugar Hill Drive, Manchester, MO 63021 (US). ADAMS, Steven, P. [US/US]; 12 Berkeley Lane, Andover, MA 01810 (US). ZUPEC, Mark, E. [US/US]; 218 Bethel Meadows Road, Caseyville, IL 62232 (US).		<b>(74) Agents:</b> KOVACEVIC, Cynthia, S. et al.; G.D. Searle & Co., Corporate Patent Department, P.O. Box 5110, Chicago, IL 60680-5110 (US).  <b>(81) Designated States:</b> AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> UREA DERIVATIVES USEFUL AS PLATELET AGGREGATION INHIBITORS  <b>(57) Abstract</b>  Novel urea derivatives are provided which inhibit platelet aggregation. This invention also pertains to pharmaceutical compositions and methods of using such derivatives.		

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UREA DERIVATIVES  
USEFUL AS PLATELET AGGREGATION INHIBITORS

Background of the Invention

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Field of the Invention

This invention pertains to urea derivatives which inhibit platelet aggregation in mammals.

Related Art

10 Fibrinogen is a glycoprotein present as a normal component of blood plasma. It participates in platelet aggregation and fibrin formation in the blood clotting mechanism.

15 Platelets are cellular elements found in whole blood which also participate in blood coagulation. Fibrinogen binding to platelets is important to normal platelet function in the blood coagulation mechanism. When a blood vessel receives an injury, the platelets binding to fibrinogen will initiate aggregation and form a thrombus.

20 Interaction of fibrinogen with platelets occurs through a membrane glycoprotein complex, known as gpIIb/IIIa; this is an important feature of the platelet function. Inhibitors of this interaction are useful in modulating or preventing platelet  
25 thrombus formation.

It is also known that another large glycoprotein named fibronectin, which is a major extracellular matrix protein, interacts with fibrinogen and fibrin, and with other structural  
30 molecules such as actin, collagen and proteoglycans. Various relatively large polypeptide fragments in the cell-binding domain of fibronectin have been found to have cell-attachment activity. (See U.S. Patents 4,517,686, 4,589,881, and 4,661,111).  
35 Certain relatively short peptide fragments from the same molecule were found to promote cell attachment

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to a substrate when immobilized on the substrate or to inhibit attachment when in a solubilized or suspended form. (See U.S. Patents 4,578,079 and 4,614,517).

5 In U.S. Patent 4,683,291, inhibition of platelet function is disclosed with synthetic peptides designed to be high affinity antagonists of fibrinogen binding to platelets. U.S. Patent 4,857,508 discloses tetrapeptides having utility as  
10 inhibitors of platelet aggregation.

Other synthetic peptides and their use as inhibitors of fibrinogen binding to platelets are disclosed by Koczewiak et al., Biochem. 23, 1767-1774 (1984); Plow et al., Proc. Natl. Acad. Sci. 82, 8057-8061 (1985); Ruggeri et al., Ibid. 83, 5708-5712 (1986); Ginsberg et al., J. Biol. Chem. 260 (7), 3931-3936 (1985); Haverstick et al., Blood 66 (4), 946-952 (1985); and Ruoslahti and Pierschbacher, Science 238, 491-497 (1987). Still  
15 other such inhibitory peptides are disclosed in EP Patent Applications 275,748 and 298,820.

U.S. Patent 4,879,313 discloses substituted alkanolic acid derivatives which inhibit protein to  
20 receptor binding and are useful for the treatment of thrombosis and cardiac infarction.

European Patent Application 372,486 discloses N-acyl beta amino acid derivatives and their salts. Said compounds are useful for  
25 inhibiting platelet aggregation in the treatment of thrombosis, stroke, myocardial infarction, inflammation and arteriosclerosis, and for inhibiting metastasis.

European Patent Application 381,033 discloses substituted alkanolic acid derivatives  
30 useful for the treatment of thrombosis, apoplexy,

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cardiac infarction, inflammation, arteriosclerosis and tumors.

European Patent Application 445,796 discloses Acetic Acid derivatives useful as a ligand for adhesive proteins on platelets. As such these compounds are useful to modulate and/or inhibit platelet aggregation.

European Patent Application 513,810 discloses amidinoaryl substituted alkanolic acid derivatives which have usefulness as inhibitors of platelet aggregation,

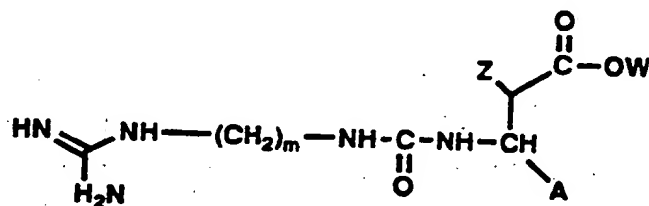
The disclosure of all references cited in this application is hereby incorporated by reference.

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#### Summary of the Invention

The present invention relates to a class of compounds represented by the formula or a pharmaceutically acceptable salt thereof:

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wherein A is the radical of the formula:



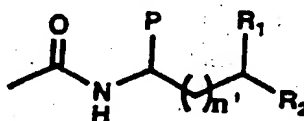
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wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals and heterocycly radicals wherein 1 to 3 heteroatoms are selected from nitrogen, oxygen or sulfur, wherein all of said radicals are optionally substituted with hydroxyl, lower alkyl, lower

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alkyl, halogen, nitro, carboxyl, trifluoromethyl, amino, acyloxy, phenyl and naphthyl which are optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl; R is preferably not substituted and when R is a heterocyclyl radical it is preferably pyrazinyl or pyridyl but more preferably pyridyl.

n is an integer from 0 to about 6;  
or A is the radical of the formula:



wherein  $R_1$  and  $R_2$  are each independently selected from hydrogen, phenyl, substituted phenyl, wherein each substituent can be selected from the group consisting of lower alkyl radicals, halogen, lower alkoxy radicals, trifluoromethyl radical, carboxyl radical, hydroxyl radical and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur; when  $R_1$  and  $R_2$  is a heterocyclyl radical it is preferably pyrazinyl or pyridyl but more preferably pyridyl.

P is hydrogen, carboxyl or alkoxycarbonyl radicals;  
n' is an integer from 0 to about 2;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkyl, lower alkoxy, halogen, nitro, amino, acyloxy, phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

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Z is independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals;

m is an integer from 5 to about 9.

The invention further relates to pharmaceutical compositions comprising a compound of Formula (I). Such compounds and compositions have usefulness as inhibitors of platelet aggregation.

It is still another object of the invention to provide a method to therapeutically inhibit or modulate platelet aggregation or the like in a mammal in need of such treatment with a compound of the formula I in unit dosage form. Particularly in inhibiting or modulating platelet aggregation by administering an amount between 0.5mg/kg to 10mg/kg, preferably 3mg/kg to an animal in need thereof.

Many other objects and purposes of the invention will be clear from the following detailed description of the invention and examples.

#### Detailed Description of the Invention

A preferred embodiment of the present invention is a compound of formula I or a pharmaceutically acceptable salt thereof; wherein A is the radical of the formula:



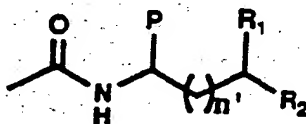
wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals

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of 3 to about 6 carbon atoms, aromatic hydrocarbon radicals and a heterocyclyl radical in which 1 to about 3 heteroatoms are independently selected from nitrogen or oxygen wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, and trifluoromethyl;

n is an integer from 0 to about 6;

or A is the radical of the formula:



wherein R<sub>1</sub> and R<sub>2</sub> are each independently selected from hydrogen, phenyl, substituted phenyl, wherein each substituent can be selected from the group consisting of lower alkyl radicals of 1 to 6 carbon atoms, halogen, lower alkoxy radicals of 1 to 6 carbon atoms, trifluoromethyl radical, carboxyl radical, hydroxyl radical and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur;

P is hydrogen, carboxyl or alkoxycarbonyl radicals of 1 to 6 carbon atoms;

n' is an integer from 0 to about 2;

W is selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6



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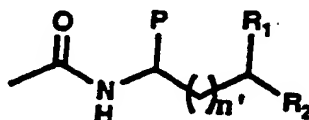
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each substituent can be selected from the group consisting of lower alkyl radicals, lower alkoxy radicals, carboxyl radical, hydroxyl radical, and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur;

P is hydrogen, carboxyl or alkoxycarbonyl radicals;

n' is an integer from 0 to about 2;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, alicyclic hydrocarbon radicals, and aromatic hydrocarbon radicals;

Z is independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, and hydroxy radicals;

m is an integer from 5 to about 9.

As utilized herein, the term "lower alkyl", alone or in combination, means an acyclic alkyl radical containing from 1 to about 10, preferably from 1 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl and the like.

The term "lower alkenyl" refers to an unsaturated acyclic hydrocarbon radical in so much as it contains at least one double bond. Such radicals containing from about 2 to about 10 carbon atoms, preferably from about 2 to about 8 carbon atoms and more preferably 2 to about 6 carbon atoms.

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Examples of suitable alkenyl radicals include propylenyl, buten-1-yl, isobutenyl, penten-1-yl, 2-2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

5           The term "lower alkynyl" refers to an unsaturated acyclic hydrocarbon radicals in so much as it contains one or more triple bonds, such radicals containing about 2 to about 10 carbon atoms, preferably having from about 2 to about 8  
10 carbon atoms and more preferably having 2 to about 6 carbon atoms. Examples of suitable alkynyl radicals include ethynyl, propynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.  
15

          The term "alicyclic hydrocarbon" means a aliphatic radical in a ring with 3 to about 10 carbon atoms, and preferably from 3 to about 6 carbon atoms. Examples of suitable alicyclic  
20 radicals include cyclopropyl, cyclopropylenyl, cyclobutyl, cyclopentyl, cyclohexyl, 2-cyclohexen-1-ylenyl, cyclohexenyl and the like.

          The term "aromatic hydrocarbon radical" means 4 to about 16 carbon atoms, preferably 6 to  
25 about 12 carbon atoms, more preferably 6 to about 10 carbon atoms. Examples of suitable aromatic hydrocarbon radicals include phenyl, naphthyl, and the like.

          The term "lower alkoxy", alone or in  
30 combination, means an alkyl ether radical wherein the term alkyl is as defined above and most preferably containing 1 to about 4 carbon atoms. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy,  
35 iso-butoxy, sec-butoxy, tert-butoxy and the like.

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The term "heterocyclyl radical" means a heterocyclyl hydrocarbon radical preferably an aromatic heterocyclyl hydrocarbon radical with 4 to about 10 carbon atoms, preferably about 5 to about 6; wherein 1 to about 3 carbon atoms are replaced by nitrogen, oxygen or sulfur. The "heterocyclyl radical" may be fused to a aromatic hydrocarbon radical or to another heterocyclyl radical. The "heterocyclyl radical" may be saturated, partially saturated, or fully unsaturated. Suitable examples include pyrrolyl, pyridinyl, pyrazolyl, pyrrolyl, triazolyl, pyrimidinyl, pyridazinyl, oxazolyl, thiazolyl, imidazolyl, indolyl, thiophenyl, furanyl, tetrazolyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolidinyl, 1,3-dioxolanyl, 2-imidazolyl, imidazolidinyl, 2-pyrazolyl, pyrazolidinyl, isoxazolyn, isothiazolyn, 1,2,3-oxadiazolyn, 1,2,3-triazolyn, 1,3,4-thiadiazolyn, 2H-pyranyl, 4H-pyranyl, piperidinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, pyrazinyl, piperazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, benzo(b)thiophenyl, benzimidazolyn, quinolinyl, and the like.

The term halogen means fluorine, chlorine, bromine or iodine.

The term "pharmaceutically acceptable salt" refers to a salt prepared by contacting a compound of formula (I), with an acid whose anion is generally considered suitable for human consumption. Examples of pharmacologically acceptable salts include the hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, propionate, lactate, maleate, oxalate, malate, succinate, tartrate and citrate salts. All of these salts may be prepared by conventional means by

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reacting, for example, the appropriate acid with the corresponding compound of formula I.

Suitable pharmaceutically-acceptable base addition salts of compounds of Formula I include  
5 metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and  
10 procaine.

Total daily dose administered to a host in single or divided doses may be in amounts, for example, from 0.001 to 100 mg/kg body weight daily and more usually 0.01 to 10 mg. Dosage unit  
15 compositions may contain such amounts of submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host  
20 treated and the particular mode of administration.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight,  
25 general health, sex, diets, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy.

The compounds of the present invention may  
30 be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Injectable preparations, for  
35 example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known

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art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperature but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions,

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solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more active pharmaceutical agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions, which are given at the same time or different times, or the therapeutic agents can be given as a single composition.

In the structures and formulas herein, the bond drawn across a bond of an aromatic ring can be to any available atom on the aromatic ring.

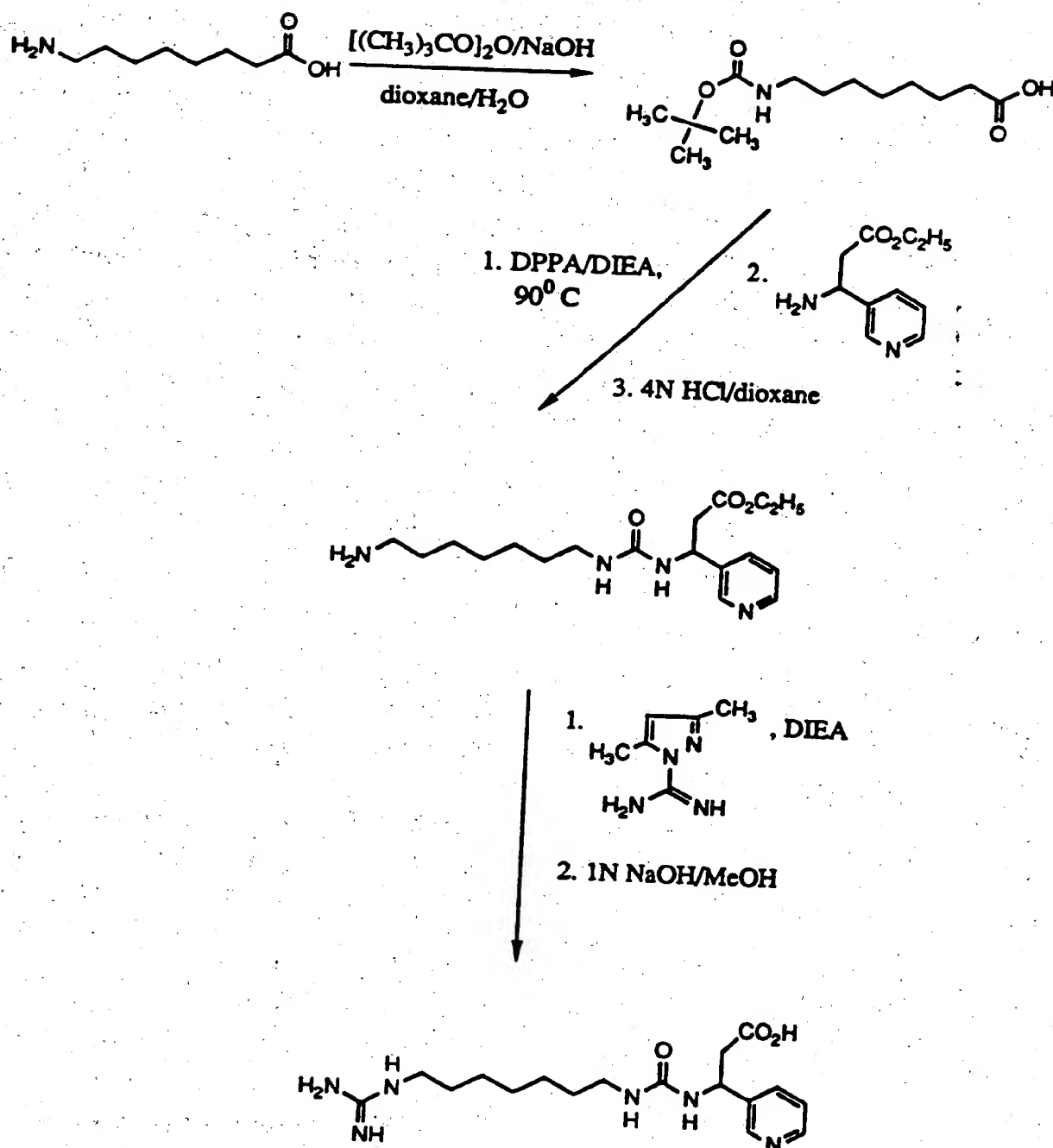
The compounds of formula I can exist in various isomeric forms and all such isomeric forms are meant to be included. Tautomeric forms are also included in the invention. Pharmaceutically acceptable salts of such isomers and tautomers are meant to be included as well.

The compounds listed above may be prepared by standard synthetic methods combined with methods analogous to solution phase peptide synthesis [see: The Peptides: Analysis, Synthesis, Biology (E. Gross and J. Meienhofer, eds.), (Vol. 1-5; Academic Press, New York)].

General synthetic sequences for preparing the compounds of formula I are outlined in scheme I and II below.

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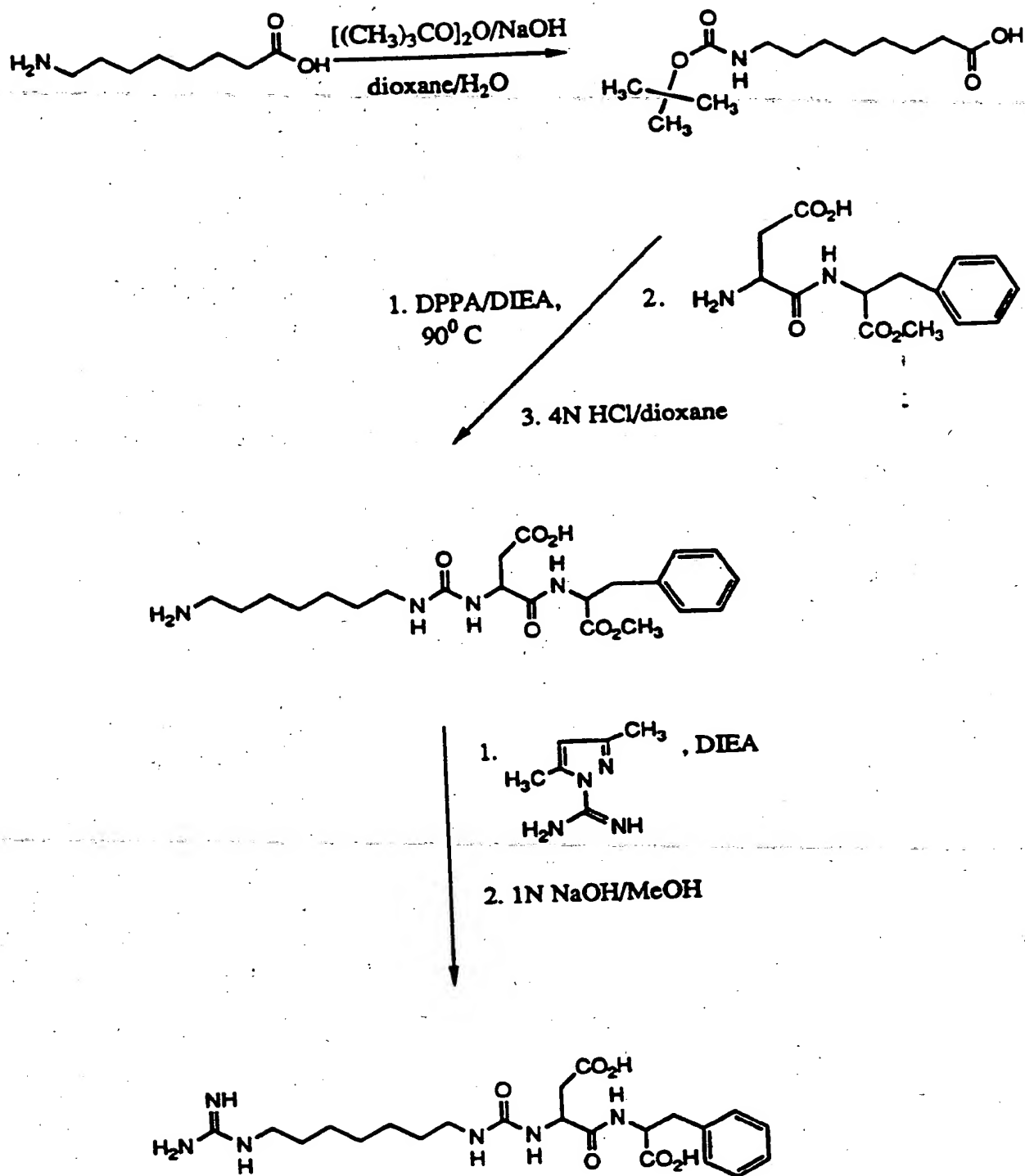
## SCHEME I





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## SCHEME II



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Purification of final compounds can be performed by reverse phase high pressure liquid chromatography [High Performance Liquid Chromatography Protein and Peptide Chemistry, F. Lottspeich, A. Henschel, K.P. Hupe, eds.) Walter DeGruyter, New York, 1981 or crystallization.

Contemplated equivalents of the general formulas set forth above for the platelet aggregation inhibitors and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general properties wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of

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reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention.

5 In all preparative methods, all starting materials are known or readily preparable from known starting materials.

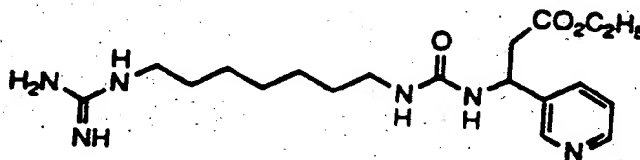
Without further elaboration, it is believed that one skilled in the art can, using the preceding  
10 description, utilize the present invention to its fullest extent. Therefore the following preferred specific embodiments are to be construed as merely, illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

15 The following examples are provided to illustrate the present invention and are not intended to limit the scope thereof. Those skilled in the art will readily understand that known variations of the conditions and processes of the  
20 following preparative procedures can be used to prepare these compounds. All temperatures expressed are in degrees centigrade.

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Example 1

Ethyl  $\beta$ -[[[7-  
[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
]3-pyridine-propanoate

**STEP 1**

8-(N-tert-butyloxycarbonyl)aminooctanoic acid

8-aminooctanoic acid (10 g; 62.9 mmoles) and di-  
tert-butyl dicarbonate (15.1g; 69.2 mmoles) were  
suspended in dioxane (20 ml) and water (4 ml).  
Sodium hydroxide (1 N) was added dropwise until the  
pH of the mixture reached 8, whereupon the solids  
dissolved. After stirring overnight, the solution  
was neutralized with 1N hydrochloric acid. The  
volume of the mixture was reduced by one half and  
the concentrated solution was placed in a  
refrigerator overnight. The solid was filtered,  
washed with a minimum of cold water and air dried to  
yield 11g of product (67% yield). FAB-MS:  $MH^+ = 260$ .

**STEP 2**

Ethyl  $\beta$ -[[[7-aminoheptyl]amino]carbonyl]amino]-3-  
pyridinepropanoate

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8-(N-tert-butyloxycarbonyl)aminooctanoic acid (2.4 g; 9.3 mmol) was dissolved in N,N-dimethylformamide (30 ml) under a nitrogen blanket and the mixture was heated to gentle reflux. N,N-Diisopropylethylamine (2.6 g; 20 mmol) and diphenylphosphoryl azide (2.75 g; 10 mmol) were added, followed by ethyl  $\beta$ -amino- $\beta$ -(3-pyridyl)-propanoate dihydrochloride (2.0 g; 8.7 mmol). The mixture was refluxed for another 4 hrs and allowed to stir at room temperature overnight. The reaction mixture was then taken down to dryness and the residue was purified by HPLC using a linear gradient of 5-45% acetonitrile/water/trifluoroacetic acid. This material was then treated with 4N hydrochloric acid/dioxane (50 ml) for 30 min. The mixture was taken down to dryness under reduced pressure to give an oily product (1.2 g). FAB-MS:  $MH^+ = 351.4$ .

## STEP 3

Ethyl  $\beta$ -[[[7-[[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-3-pyridinepropanoate

Ethyl  $\beta$ -[[[7-aminoheptyl]amino]carbonyl]amino]-3-pyridinepropanoate (1.2 g; 3 mmol), 3,5-dimethylpyrazole-1-carboxamide nitrate (2.6 g; 13.3 mmol) and diisopropylethylamine (3.74 g; 28.7 mmol) were stirred in dioxane (25 ml) and water (10 ml) for 22 hrs. The mixture was taken down to dryness under reduced pressure and the residue was purified by HPLC using a linear gradient of 5-45% acetonitrile/water/trifluoroacetic acid. The desired fractions were collected and lyophilized to give 875 mg of clear oily material. FAB-MS:  $MH^+ = 393.2$ .

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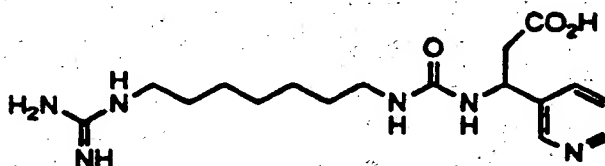
Elemental analysis:  $C_{19}H_{32}N_6O_3 \cdot CF_3COOH \cdot 4H_2O$

	C	H	N
Calculated:	43.63	7.14	14.53
Found:	43.34	7.35	15.69

### Example 2

$\beta$ -[[[[7-

[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
]-3-pyridinepropanoic acid



A portion of ethyl  $\beta$ -[[[[7-  
[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
]-3-pyridinepropanoate (310 mg) was treated with 1 N  
sodium hydroxide and methanol (1:1; 20ml) for 5 min.  
Methanol was removed under reduced pressure and the  
residue was purified by HPLC using a linear gradient  
of 5-45% acetonitrile/water/trifluoroacetic acid.  
The desired fractions were collected and lyophilized  
to give 280 mg of white material. FAB-MS :  $MH^+ =$   
365.1.

Elemental analysis:  $C_{17}H_{28}N_6O_3 \cdot 2CF_3COOH$

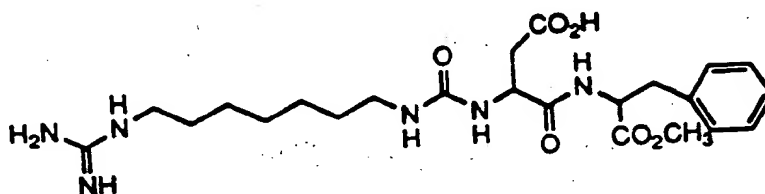
	C	H	N
Calculated:	42.57	5.10	14.18
Found:	42.35	5.13	14.12

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Exempl 3

N-[N-[[[7-  
 5 [[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
 ]-L- $\alpha$ -aspartyl]-L-phenylalanine methyl ester

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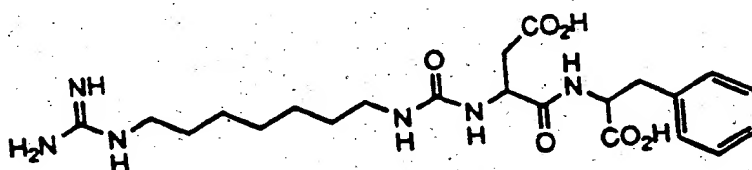
15 8-guanidinooctanoic acid.HCl (1.25 g; 5 mmoles) was  
 dissolved in N,N-dimethylformamide (50 ml) and  
 heated up to about 80°C under a nitrogen blanket.  
 To this hot solution, N,N-diisopropylethylamine  
 (1.30 g; 10 mmoles) and diphenylphosphoryl azide  
 20 (1.38 g; 5mmoles) were added dropwise. After 10  
 min., a suspension of L- $\alpha$ -aspartyl-L-phenylalanine  
 methyl ester (1.47 g; 5 mmoles) in N,N-  
 dimethylformamide was added with vigorous stirring.  
 The clear reaction mixture was stirred at room  
 25 temperature overnight and taken down to dryness  
 under reduced pressure. The residue was partially  
 purified by HPLC using a linear gradient of 5-60%  
 acetonitrile/water/trifluoroacetic acid. The  
 desired fractions were collected and lyophilized to  
 30 give 410 mg of white material.  
 FAB-MS: MH<sup>+</sup> = 492.

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Exempl 4

5 N-[N-[[[7-  
 [[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
 ]-L- $\alpha$ -aspartyl]-L-phenylalanine

10



15

A portion of N-[N-[[[7-  
 [[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino  
 ]-L- $\alpha$ -aspartyl]-L-phenylalanine methyl ester (100  
 mg) was treated with 2 N lithium hydroxide and  
 20 methanol (1:1; 20ml) for 15 min. Methanol was  
 removed under reduced pressure and the residue was  
 purified by HPLC using a linear gradient of 10-60%  
 acetonitrile/water/trifluoroacetic acid. The  
 desired fractions were collected and lyophilized to  
 25 give 35 mg of white material. FAB-MS :  $MH^+$  = 479.  
 $^1H$ -NMR (DMSO- $d_6$ )  $\delta$  1.2-1.5 (m, 10 H,  $CH_2-(CH_2)_5-CH_2$ ),  
 2.40 (m, 2H,  $CH_2-(CH_2)_5-\underline{CH_2}$ ), 2.57 (m, 2H, Asp  $\beta$ -  
 $CH_2$ ), 2.97 (m, 2H,  $CH_2-(CH_2)_5-CH_2$ ), 3.04 (m, 2H, Phe  
 $\beta-CH_2$ ), 4.02 (br, 1H, Phe  $\alpha-CH_2$ ), 4.41 (br, 1H,  
 30 Asp  $\alpha-CH_2$ ), 6.10 (m, 2H, NH-CO-NH), 7.10 (m, 5H,  
 aromatic), 7.2 and 7.90 (guanidinium NH).



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Further examples prepared by similar methods described above are the following examples:

5

Example 5

N-[N-[[[6-  
[[aminoiminomethyl]amino]hexyl]amino]carbonyl]amino]-  
-L- $\alpha$ -aspartyl]-L-phenylalanine;  
FAB-MS :  $MH^+$  = 465.

10

Example 6

N-[N-[[[7-[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-L-  
 $\alpha$ -aspartyl]-L-tryptophan.  
FAB-MS :  $MH^+$  = 518.

15

In-Vitro Platelet Aggregation in PRP

Healthy male or female dogs were fasted for 8 hours prior  
20 to drawing blood; then 30 ml whole blood was collected using a  
butterfly needle and 30 cc plastic syringe with 3 ml of 0.129 M  
buffered sodium citrate (3.8%). The syringe was rotated carefully as  
blood was drawn to mix the citrate. Platelet-rich plasma (PRP) was  
prepared by centrifugation at 975 x g for 3.17 minutes at room  
25 temperature allowing the centrifuge to coast to a stop without  
braking. The PRP was removed from the blood with a plastic pipette  
and placed in a plastic capped, 50 mL Corning conical sterile  
centrifuge tube which was held at room temperature. Platelet poor  
plasma (PPP) was prepared by centrifuging the remaining blood at  
30 2000 x g for 15 minutes at room temperature allowing the centrifuge  
to coast to a stop without braking. The PRP was adjusted with PPP  
to a count of  $2-3 \times 10^8$  platelets per mL. 400 uL of the PRP  
preparation and 50 uL of the compounds solution to be tested or

saline were preincubated for 1 minute at 37°C in a BioData, Horsham, PA). 50 uL of adenosine 5' diphosphate (ADP) (50 um final concentration) was added to the cuvettes and the aggregation was monitored for 1 minute. All compounds are tested in duplicate. Results are calculated as follows: Percent of control = [(maximal OD minus initial OD of compound) divided by (maximal OD minus initial OD of control saline)] x 100. The % inhibition = 100 - (percent of control).

The compounds tested and their median inhibitory concentrations (IC<sub>50</sub>) are recorded in Table I. IC<sub>50</sub>'s (dosage at which 50% of platelet aggregation is inhibited) were calculated by linear regression of the dose response curve. The assay results for the compounds of Examples 1 to 4 are set forth in Table I below.

#### INHIBITION OF EX VIVO COLLAGEN INDUCED AGGREGATION

##### BY COMPOUNDS OF THE INVENTION

The purpose of this assay is to determine the effects of antiplatelet compounds on ex vivo collagen induced platelet aggregation when administered either intravenously or orally to dogs.

Pretreatment (control) blood samples are drawn from either conscious or anesthetized dogs (Beagles) and centrifuged to prepare platelet rich plasma (PRP). Aggregatory response to collagen is measured in a aggregometer and used as Control. Compounds are administered, either intragasterically (either by capsule or stomach tube or intravenously). Blood samples are drawn at predetermined intervals after compound administration, PRP prepared and aggregation to collagen determined. Compound inhibition of aggregation is determined by comparing the aggregation response after compound administration to the pretreatment response.

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Table I

5	Example	Dog PRP IC <sub>50</sub> or % Inh.	Ex Vivo Effect after IG Administration
10	1	NT	+
	2	8.0 X 10 <sup>-7</sup> M	NT
	3	NT	NT
	4	1.9 X 10 <sup>-6</sup> M	NT

15

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NT - not tested

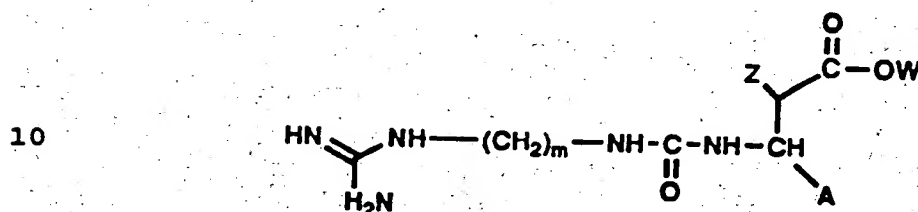
20

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various

25 changes and modifications of the invention to adapt it to various usages and conditions.

WHAT IS CLAIMED IS:

1. A compound or a pharmaceutically acceptable salt thereof  
 5 having the formula:



15 wherein A is the radical of the formula:



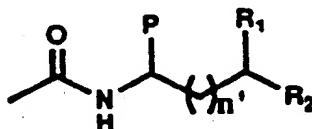
20 wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, aromatic hydrocarbon radicals and heterocycly radicals wherein 1 to 3 hetroatoms are selected from nitrogen, oxygen or sulfur, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, trifluoromethyl, amino, acyloxy, phenyl and naphthyl which  
 25 are optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

n is an integer from 0 to about 6;

30

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or A is the radical of the formula:



wherein  $\text{R}_1$  and  $\text{R}_2$  are each independently selected from hydrogen, phenyl, substituted phenyl, wherein each substituent can be selected from the group consisting of lower alkyl radicals, halogen, lower alkoxy radicals, trifluoromethyl radical, carboxyl radical, hydroxyl radical and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur;

P is hydrogen, carboxyl or alkoxycarbonyl radicals;

$n'$  is an integer from 0 to about 2;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkyl, lower alkoxy, halogen, nitro, amino, acyloxy, phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

Z is independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals; and

m is an integer from 5 to about 9.

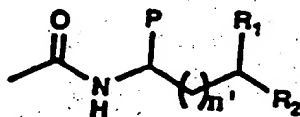
2. A compound according to claim 1  
wherein A is the radical of the formula:



wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, aromatic hydrocarbon radicals and a heterocyclyl radical in which 1 to about 3 heteroatoms are independently selected from nitrogen or oxygen wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, and trifluoromethyl;

n is an integer from 0 to about 6;

or A is the radical of the formula:



wherein  $R_1$  and  $R_2$  are each independently selected from hydrogen, phenyl, substituted phenyl, wherein each substituent can be selected from the group consisting of lower alkyl radicals of 1 to 6 carbon atoms, halogen, lower alkoxy radicals of 1 to 6 carbon atoms, trifluoromethyl radical, carboxyl radical, hydroxyl radical and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur;

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P is hydrogen, carboxyl or alkoxycarbonyl radicals of 1 to 6 carbon atoms;

n' is an integer from 0 to about 2;

5

W is selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals of 6 to about 12 carbon atoms, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, and acyloxy;

10

Z is independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals; and

15

m is an integer from 5 to about 9.

20

3. A compound according to claim 1 wherein A is the radical of the formula:



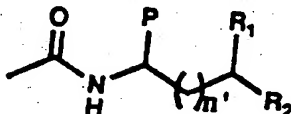
25

wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals and aromatic hydrocarbon radicals, or R is a heteromonocyclic ring structure having 5 or 6 ring carbon atoms wherein 1 or 2 of the ring carbon atoms are replaced by nitrogen or oxygen;

30

n is an integer from 0 to about 6;

or A is the radical of the formula:



wherein  $\text{R}_1$  and  $\text{R}_2$  are each independently selected from hydrogen, phenyl, substituted phenyl, wherein each substituent can be selected from the group consisting of lower alkyl radicals, lower alkoxy radicals, carboxyl radical, hydroxyl radical, and a 5- or 6-membered heterocyclic ring alone or fused to a benzene ring wherein the hetero atom is selected from nitrogen, oxygen and sulfur;

P is hydrogen, carboxyl or alkoxycarbonyl radicals;

$n'$  is an integer from 0 to about 2;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, alicyclic hydrocarbon radicals, and aromatic hydrocarbon radicals;

Z is independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, and hydroxy radicals; and

m is an integer from 5 to about 9.



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4. A compound according to claim 1 which is selected from the group consisting of:

Ethyl  $\beta$ -[[[[7-  
5 [[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]3-pyridine-  
propanoate;

$\beta$ -[[[[7-[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-3-  
pyridine-propanoic acid;

10 N-[N-[[[[7-[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-  
L- $\alpha$ -aspartyl]-L-phenylalanine methyl ester;

N-[N-[[[[7-[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-  
15 L- $\alpha$ -aspartyl]-L-phenylalanine;

N-[N-[[[[6-[[aminoiminomethyl]amino]hexyl]amino]carbonyl]amino]-L-  
 $\alpha$ -aspartyl]-L-phenylalanine; and

20 N-[N-[[[[7-[[aminoiminomethyl]amino]heptyl]amino]carbonyl]amino]-  
L- $\alpha$ -aspartyl]-L-tryptophan.

5. A pharmaceutical composition comprising at least one non-toxic  
pharmaceutically acceptable carrier and at least one compound  
according to Claim 1 together with said carrier.

25 6. A pharmaceutical composition comprising at least one non-toxic  
pharmaceutically acceptable carrier and at least one compound  
according to Claim 2 together with said carrier.

7. A pharmaceutical composition comprising at least one non-toxic  
pharmaceutically acceptable carrier and at least one compound  
30 according to Claim 3 together with said carrier.

8. A pharmaceutical composition comprising at least one non-toxic pharmaceutically acceptable carrier and at least one compound according to Claim 4 together with said carrier.
9. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claim 1 to a mammal in need of such treatment.
10. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claim 2 to a mammal in need of such treatment.
11. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claim 3 to a mammal in need of such treatment.
12. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claim 4 to a mammal in need of such treatment.

## INTERNATIONAL SEARCH REPORT

Intern al Application No  
PCT/US 94/02157A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 C07C279/12 A61K31/44 C07D213/55

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 526 877 (BRISTOL-MYERS SQUIBB COMPANY) 10 February 1993 ----	
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 263, no. 11, 1988, BALTIMORE, MD US pages 5210 - 5216 L.F. BRASS 'Inhibition of thrombin-induced platelet activation by leupeptin' see page 5210, left column -----	

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*I\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

13 June 1994

Date of mailing of the international search report

- 4. 07. 94

Name and mailing address of the ISA

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Authorized officer

De Jong, B

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/02157

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 9-12 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the of compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/02157

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0526877	10-02-93	NONE	

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